816
ACCUMULATION OF CD4+CD25+/HIGHCD127LOW/- REGULATORY T CELLS IN OSTEOARTHRITIS JOINTS – ANALYSIS OF FREQUENCY AND PHENOTYPE IN SYNOVIAL MEMBRANE, SYNOVIAL FLUID AND PERIPHERAL BLOOD

B. Moradi [†], P. Schnatzer [†], S. Hagmann [†], N. Rosshirt [†], T. Gotterbarm [†], H-M. Lorenz [‡], T. Tretter [‡], F. Zeifang [†]. [†] *Univ. Clinic of Heidelberg, Dept. of Orthopaedics and Trauma Surgery, Heidelberg, Germany;* [‡] *Univ. Clinic of Heidelberg, Dept. of Med. V, Div. of Rheumatology, Heidelberg, Germany*

Purpose: Osteoarthritis (OA) is the most common joint disease and up to date there are no therapies to halt disease initiation or progression. It is becoming more evident that all other joint structures are affected in OA and that the progressive pathological changes in the joint tissues perpetuate disease progression. The synovial membrane (SM) functions as an important site for this complex cell interaction and the molecular interplay. There is an increasing body of evidence that synovitis in OA may not just result from, but also play an active role in disease onset and progression. This could lead to a disturbed immune regulation in OA joints with the predominance of inflammatory cells on one hand and a defective peripheral immune tolerance on the other.
Naturally occurring regulatory T cells (Treg), have been shown to play an essential role in establishing the balance between pro- and anti-inflammatory mechanisms in the periphery and maintaining self-tolerance both in rodents and humans. Their ability to suppress T cell responses and thereby regulate immune reactions ascribes them a key role in the pathophysiology of inflammation and makes them an interesting target for treatment. The presence of Tregs in OA joints and their functional status has not been assessed yet.
In this study we aimed to investigate the presence of Tregs in OA patients and to map their distribution between periphery and the target organ. Data were put in relation to resting T cells and activated effector T cells. Further, we assessed functional differences between peripheral and synovial Tregs.
Methods: Data on 44 OA patients were prospectively collected. The presence and phenotype of CD4+CD25- naive (Tn), CD4+CD25+ CD127+ activated effector (Teff) and CD4+CD25+/highCD127low/- Tregs were assessed in concurrent samples of synovial membrane, synovial fluid (SF) and peripheral blood (PB) by multicolor flow cytometry analysis. Further, we analyzed functional differences between peripheral and synovial Tregs by surface markers representative of activation (CD45RA, CD45R0, CD69, CD62L), memory and regulation (CD152, CD154, CD223, CD274, CD279, GITR).
Results: The synovial membrane contained a significantly higher concentration of infiltrating CD4+ T cells in comparison to SF. A significant change of CD4+ T cell subsets was shown in the joint samples when compared to PB, displaying the same pattern for SF and SM. CD4+CD25+/highCD127low/- Tregs showed a significant accumulation into the synovial fluid (14.9 ± 4 % of CD4+ T cells) and membrane (12 ± 5) when compared to concurrent PB samples (8.4 ± 4.2; $p < 0.05$). In SF a significant decrease of CD4+CD25- Tns (23 ± 15) was found when compared to PB (40 ± 9.2; $p < 0.05$) without any significant change in CD4+CD25+CD127+ Teffs (PB: 39.3 ± 8.5; SF 42 ± 11; n.s.). In SM the decrease of CD4+CD25- Tns was even more evident (14 ± 11.5, $p < 0.001$). Here, the percentage of CD4+CD25+CD127+ Teffs (OA: 51 ± 17) was significantly increased compared to concurrent PB samples (OA: 39.3 ± 8.5; $p < 0.05$). Both, PB and SM Tregs displayed a memory phenotype (CD45RO+RA-) but synovial Tregs were activated effector memory cells (CD62L-CD69+), while peripheral Tregs were resting central memory cells (CD62L+CD69-). Further, PB and SM Tregs significantly differed in markers associated with Treg function (CD152, CD154, CD274, CD279, GITR).
Conclusions: This is, to the authors' best knowledge, the first study to show that Tregs are enriched in OA joints. One underlying hypothesis about Treg enrichment into the affected joints of inflammatory diseases is that Tregs are either attracted or generated due to on-going inflammation. We further detected a significant increase of Teffs in the SM. This taken into account, the accumulated Treg presence in the joints could be understood as an attempt of the immune system to control the inflammatory responses. Further, we showed that synovial Treg show significant increase of activation markers and markers associated with Treg function suggesting that synovial Tregs are active and contribute to joint homeostasis. Increasing Treg mediated suppressive capacity in the joints could provide a new therapeutic approach in OA treatment.

817
A SPLICE VARIANT OF ADAMTS4 IS EXPRESSED AS A PROTEIN IN HUMAN OSTEOARTHRITIS SYNOVIUM AND CLEAVES AGGRECAN AT THE INTERGLOBULAR DOMAIN

S.D. Wainwright [†], J. Bondeson [‡], B. Caterson [†], C.E. Hughes [†]. [†] *Cardiff Sch. of BioSci.s, Cardiff, United Kingdom;* [‡] *Dept of Rheumatology, Cardiff Univ., Cardiff, United Kingdom*

Purpose: Osteoarthritis (OA) is a multifaceted disease involving pathological changes in the articular cartilage, subchondral bone, surrounding soft tissues, and synovium. Inflammation and proliferation of synovium occurs in both early and late stage OA, and the synovium produces many proinflammatory cytokines and destructive enzymes. OA synovium is known to produce the ADAMTS4 and ADAMTS5 metalloproteinases, key enzymes in the cleavage of aggrecan and degradation of cartilage. Aggrecan degradation is a crucial early event in the pathogenesis of OA, and studies have found evidence for the involvement of either ADAMTS4 or ADAMTS5, or both these enzymes, in the progression of OA.
We have previously described a mRNA variant of ADAMTS4 (ADAMTS4_v1) in human synovial cell co-cultures obtained from OA patients. This RNA message has only been found in OA synovium, and not in OA cartilage or other tissues. If translated, it would result in a protein identical to ADAMTS4, except that the C-terminal spacer domain would be completely different, without homologies to ADAMTS4. Thus it would be predicted to retain enzyme specificity for aggrecan degradation, although properties dependent on the ADAMTS4 spacer domain would be lost. The purpose of this study was to determine if ADAMTS4_v1 is translated into a protein, expressed in vivo, and capable of acting as an aggrecanase and cleaving other matrix proteoglycans.
Methods: Polyclonal antibodies were raised against synthetic peptides representing unique amino acid sequences in the C-terminus of ADAMTS4_v1 protein. After these antibodies had been characterised, an immunohistochemical study of human OA synovium was performed. A mammalian expression vector coding for FLAG-tagged human ADAMTS4 was mutated to contain the different sequence of ADAMTS4_v1 and the resultant plasmid was used to transfect HEK293 cells. ADAMTS4_v1 protein produced by these cells was purified via the FLAG epitope, and the ability of this recombinant protein to cleave aggreacan, biglycan and decorin was investigated.
Results: An antibody specific for ADAMTS4_v1 protein was found to bind to the synovial membrane surface on cryosections of human OA synovium. The staining was most prominent in the superficial layer of the synovial membrane. The ADAMTS4_v1 protein could be detected in cell lysates from OA synovial samples. The recombinant ADAMTS4_v1 protein demonstrated enzyme activity towards the target substrate in a commercial aggrecanase 1 enzyme-linked immunosorbent assay. It was also found to cleave aggrecan at the pathologically important Glu373 ↓ 374Ala aggrecanase site, and to cleave biglycan but not decorin.
Conclusions: The splice variant of ADAMTS4 (ADAMTS4_v1) is expressed as a protein in vivo in human OA synovium, functions as an aggrecanase, and cleaves other proteoglycan substrates. This splice variant may be an important contributor to the loss of aggrecan from the superficial zone of OA cartilage.

818
THE GLUCOCORTICOID-INDUCED LEUCINE ZIPPER (GILZ) PROTEIN IS INVOLVED IN CORTICOSTEROID-INDUCED LEPTIN PRODUCTION BY HUMAN OSTEOARTHRITIS SYNOVIAL FIBROBLASTS IN VITRO

O. Malaise, B. Relic, E. Charlier, S. Neuville, D. de Seny, M. Malaise. *GIGA Res. – Univ. of Liège – CHU Liège, Liège, Belgium*

Purpose: Osteoarthritis (OA) can be considered as a metabolic disorder and adipokine leptin is involved in osteoarthritis pathogenesis: leptin synovial levels are higher in patients with OA knee than in control subjects and leptin synovial and serum levels are correlated to the radiologic severity of OA knee. We have previously shown that synovial fibroblasts themselves were able to produce leptin in vitro, with a strong induction under glucocorticoid prednisolone treatment. Glucocorticoids are powerful anti-inflammatory drugs and glucocorticoid-induced leucine zipper (GILZ) mediates parts of its anti-inflammatory effect. In collagen-induced arthritis and in rheumatoid arthritis synovial fibroblasts, GILZ shows an anti-inflammatory action. On the other hand,

glucocorticoids have also serious long-term adverse events, as mellitus diabetes and osteoporosis.
GILZ is involved in osteogenic differentiation and reduces adipogenic differentiation of mesenchymal stem cells. Previously, we have shown that genistein promoted adipogenesis, but inhibited leptin production. In regards to theses observations, the aim of this study was to determine GILZ's implication in corticosteroid-induced leptin secretion.
Methods: Human synovial fibroblasts were isolated from OA patients during knee surgery. Lentiviral vectors were generated by co-transfecting Lenti-X 293T cells (Clonetech, Belgium) with a pSPAX2 plasmid (Addgene, Plasmid #12260), a VSV-G encoding vector and a GILZ (TSC22D3) shRNA plasmid (#TRCN0000013793 (shRNA1), #TRCN0000 364625 (shRNA2) or #TRCN0000369187 (shRNA3), Sigma-Aldrich, USA) or with a non target sequence encoding plasmid (Sigma, Belgium, SHC002). Cells were treated with prednisolone, aldosterone, Compound A, mifepristone, eplerenone and spironolactone. ELISA measured leptin production in culture supernatant. GR, GILZ and GAPDH were analyzed in total cell extracts by Western Blot.
Results: 1. Glucocorticoid (GR) and mineralocorticoid (MR) receptors were both expressed by OA synovial fibroblasts. Glucocorticoid prednisolone induced GILZ expression in OA synovial fibroblasts. Mineralocorticoid aldosterone also induced GILZ expression, but in a lower extend. Both GILZ expressions were dependent on GR. Indeed, GR antagonist (mifepristone), but not MR antagonists (eplerenone and spironolactone), inhibited prednisolone- and aldosterone-induced GILZ expressions. Previously, we observed similar modulation for leptin secretion, with a stimulation by prednisolone and aldosterone, through GR but not MR.
2. GILZ expression was already observed by western-blot after 24 h-stimulation with prednisolone. Under prednisolone stimulation, leptin secretion gradually increased day after day, with a similar profile of expression than GILZ, hypothesizing a link between GILZ and leptin expression. Opposite to prednisolone, Compound A, a dissociative glucocorticoid receptor agonist, did not induce neither leptin nor GILZ expression in human OA SF. Theses observations suggested a link between GILZ and leptin expression.
3. Upon prednisolone stimulation, GILZ-shRNA reduced GILZ expression with 3 different shRNA. When GILZ was down regulated, prednisolone-induced leptin was significantly decreased compared to the controls. Decrease of leptin expression was correlated to the degree of GILZ extinction. GR down-regulation was not affected by GILZ-silencing after seven days of stimulation.
Conclusions: Leptin and GILZ's modulations shared similar properties. GILZ silencing led to a significantly decrease expression of leptin under prednisolone stimulation. These results suggest that GILZ could be involved in prednisolone-induced leptin in OA synovial fibroblasts.

819
PIGMENTED VILLONODULAR SYNOVITIS (PVNS)- LIKE CHANGES IN PERIPROTHETIC INTERFACE MEMBRANES

S. Soeder, A. Hartmann, A. Agaimy. *Univ. of Erlangen and Nuernberg, Erlangen, Germany*

Purpose: PVNS is a tumour-like mesenchymal lesion of uncertain histogenesis that may occur at both intra-articular and extra-articular sites. There have been infrequent reports about PVNS or similar changes after total endoprothesis (TEP). In this study, we describe and quantify the occurrence of PVNS-like lesions in the neosynovia following total endoprothesis.
Methods: Periprothetic membrane specimens from 477 patients undergoing replacement of their knee TEP were paraffin embedded, sectioned and hematoxylin-eosin stained. For evaluation of iron deposits the classic Prussian blue reaction was used. Wear particles were detected by conventional and polarisation microscopy. In order to compare PVNS-like lesions with genuine PVNS, 10 cases of the collective of PVNS-like periprothetic membranes as well as 10 representative PVNS cases were stained with antibodies against CD3, CD4, CD8, CD20, CD34, CD68, CD138, mast cell tryptase (MCT), collagen IV and Mib-1/Ki67.
Results: The neosynovia displayed a broad spectrum of histological changes. In 5,0 % (24 of 477) of all periprothetic membranes we found histological lesions showing some features of PVNS. In 3,8 % (18 of 477) of the cases the histology closely resembled PVNS with marked villous hyperplasia, fibrosis, iron deposits and a mixed cellular infiltrate composed of mononuclear cells, fibroblasts, foam histiocytes and multinucleated giant cells with varying hemosiderin deposits. Wear particles were detected in 63,3 % (302 of 477) of all perprothetic membranes and in 100% of the cases with features of PVNS (p-value <0,002). Regarding the age of the patients, no statistically significant difference was found between neosynovia with and without features of PVNS (69,4 +/-11,3 and 67,5 +/-11,3 respectively). The elapsed time between primary arthroplasty and revision surgery was 9,9 +/-6,4 years, a typical value for periprothetic membranes of the wear type. Immunohistochemistryshowed a similar pattern for PVNS and PVNS-like lesions, with a lower positivity for B- and T-Cells and especially for cytotoxic T-cells in PVNS-like lesions.
Conclusions: PVNS- like lesions found in the neosynovia of periprothetic membranes represent an exuberant fibrohistiocytic reaction and are likely induced by wear particles. Their development might be governed by the same pathological principle responsible for the classical PVNS in synovial membranes, probably involving chronic inflammatory/ irritation-induced synovial hyperplasia. These findings might provide an additional clue for the understanding of the pathological mechanism of classical PVNS.

820
SYNOVIAL TISSUE EXHIBITS MOLECULAR HETEROGENEITY IN RESPONSE TO INTRAARTICULAR KNEE JOINT SURGERY

K.I. Barton, B.J. Heard, N.M. Solbak, M. Chung, Y. Achari, N.G. Shrive, D.A. Hart, C.B. Frank. *Univ. of Calgary, Calgary, AB, Canada*

Purpose: Severe injury to the knee joint, such as anterior cruciate ligament (ACL) tears and/or menisci damage often results in accelerated osteoarthritis (OA). It is thought that there may be an injury-induced, mechanical abnormality of the injured joint and subsequent interplay between altered mechanics and biological changes, such as inflammation, which may lead to OA. In recent years, the role of synovium in the pathophysiology of OA has gained importance. Clinical evidence suggests that inflamed synovium can add to adjacent articular cartilage damage, potentially contributing to OA. Histological analysis of synovial tissue biopsies from OA patients has demonstrated abnormalities: thickening, increased vascularity, and inflammatory infiltration. We have previously demonstrated that normal synovial tissue is a homogenous tissue for biomarkers of inflammation. However, following ACL reconstruction (ACL-R), the synovium exhibits increased mRNA levels for select inflammatory markers. Currently, it is unknown whether inflammation in the synovium tissue is uniformly distributed. Microscopic observation of synovium for pathological changes may provide relevant prognostic information, which can help in the early identification of the disease and possibly influence the therapeutic decisions. The hypothesis was that synovial tissue from different locations of the knee joint would demonstrate a uniform pattern in inflammatory marker expression.
Methods: Six skeletally mature, female Suffolk Cross sheep were allocated into three groups: ACL-R ($n = 2$), surgical sham ($n = 2$), and non-operated controls ($n = 2$). Surgeries involved an arthrotomy to the right stifle joint. For the ACL-R, the stifle joint was opened on the lateral side and the patella was dislocated medially to expose the ACL. A dry pneumatic drill was fitted over the guide pin and used to core out the uninjured ACL on its femoral insertion. The bone core was then released and immediately fixed in place using two crossed Kirschner wires. For the surgical sham, a similar arthrotomy and surgical approach to the ACL-R was used. The stifle joint was opened on the lateral side and the patella was dislocated medially to expose the ACL, followed by a similar standard closure. There was no injury to the ligaments in sham animals. The control group did not have the intervention. Animals were sacrificed 2 weeks after surgery and synovium tissue was collected from four different locations (Fig. 1), and snap frozen for mRNA analysis using real time qPCR. Part of the synovium was fixed for histological analysis and evaluated for microscopic changes with Hematoxylin and Eosin staining. ANOVA with Bonferroni post-hoc analysis was used to determine differences in mRNA expression between groups, using SPSS 19.0.
Results: Analysis of the synovium tissue exhibited that pro-inflammatory interleukins (IL) were generally elevated in the operated animals but not in the shams or non-operated controls. IL-8 and IL-1β were found to be distinctly higher in the lateral region of the synovium (Fig. 2A-B). Furthermore, matrix metalloproteinase (MMP)-13 was found to be higher in the lateral region (Fig. 2C). In contrast, IL-6 mRNA expression levels in the four locations of the synovium was similar (Fig. 2D). The surgical sham animals did not exhibit the same response as the ACL-R animals. Histological observations of the four locations of